

Determination of Butylated Hydroxyanisole and Butylated Hydroxytoluene in Potato Flakes

Anglin, Mahon, and Chapman (1) developed a method for the determination of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in edible fats. A modification of this method is satisfactory for the analysis of potato flakes containing these two antioxidants. Essentially, the method consists of determining the BHA by reaction with Gibbs' reagent (3), the total antioxidant content by the Emmerie-Engel method (2), and obtaining the BHT by difference.

In the Emmerie-Engel method, the phenolic antioxidants react with ferric chloride solution in the presence of 2,2'-bipyridine. The ferrous ion which results from reduction of the ferric ion by the antioxidant complexes with the 2,2'-bipyridine to give a characteristic red color. Gibbs' reagent (2,6-dichloroquinonechloroimide) couples with phenolics in the *ortho* or *para* position to form indophenols having a characteristic blue color. Consequently, BHA (2- and 3-*tert*-butyl-4-hydroxyanisole) will react, whereas BHT (3,5-di-*tert*-butyl-4-hydroxytoluene), being blocked in the reactive positions, will not.

Method Development

These phenolic antioxidants can be isolated from dehydrated potatoes by steam distillation of an aqueous slurry of the flaked product which normally is slightly acidic (about pH 6). However, sodium sulfite is added in the processing of potato flakes and the presence of sulfur dioxide in the distillate will interfere with both methods of analysis, causing low values by the Gibbs method and high values by the Emmerie-Engel procedure. Distillation from a medium sufficiently alkaline to retain the sulfur dioxide (pH 8-10) results in a positive interference in the BHA determination. The use

of a scrubbing solution in the trap (Fig. 1) which is sufficiently alkaline to absorb the sulfur dioxide but not so basic as to remove the phenolics overcomes this problem. An aqueous suspension of magnesium oxide is recommended because it requires neither exact measurement of reagent nor adjustment of pH.

The reaction between BHT and ferric chloride is not completed in the time allotted by the procedure described; therefore accurate time control is necessary. The color development is also sensitive to temperature and light, so the sample and reagents must be adjusted to room temperature before mixing and the solution must be protected from light. The reaction between BHA, or a mixture of the two antioxidants, and ferric chloride behaves similarly. In addition, it appears that the presence of BHA accelerates the reaction rate of BHT without affecting the rate of BHA reduction of ferric ion. This conclusion is based on data (taken at constant temperature) presented in Table 1. In every instance, the absorbance of a solution containing both BHA and BHT is greater than the sum of the absorbances of each component. With BHA alone, Beer's law is obeyed, while with BHT alone, it is not.

Table 1. Absorbances of BHA and BHT solutions by the Emmerie-Engel method

Mmg BHT	Mmg BHA				
	0	4	12	20	A*
0	—	.026	.076	.128	—
4	.016	.058	.109	.161	.033
12	.052	.117	.174	.224	.095
20	.100	.182	.243	.290	.162

* Average absorbance of BHT in the presence of BHA, obtained by subtracting BHA absorbances (line 1) from absorbances for solutions containing both antioxidants.

* Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

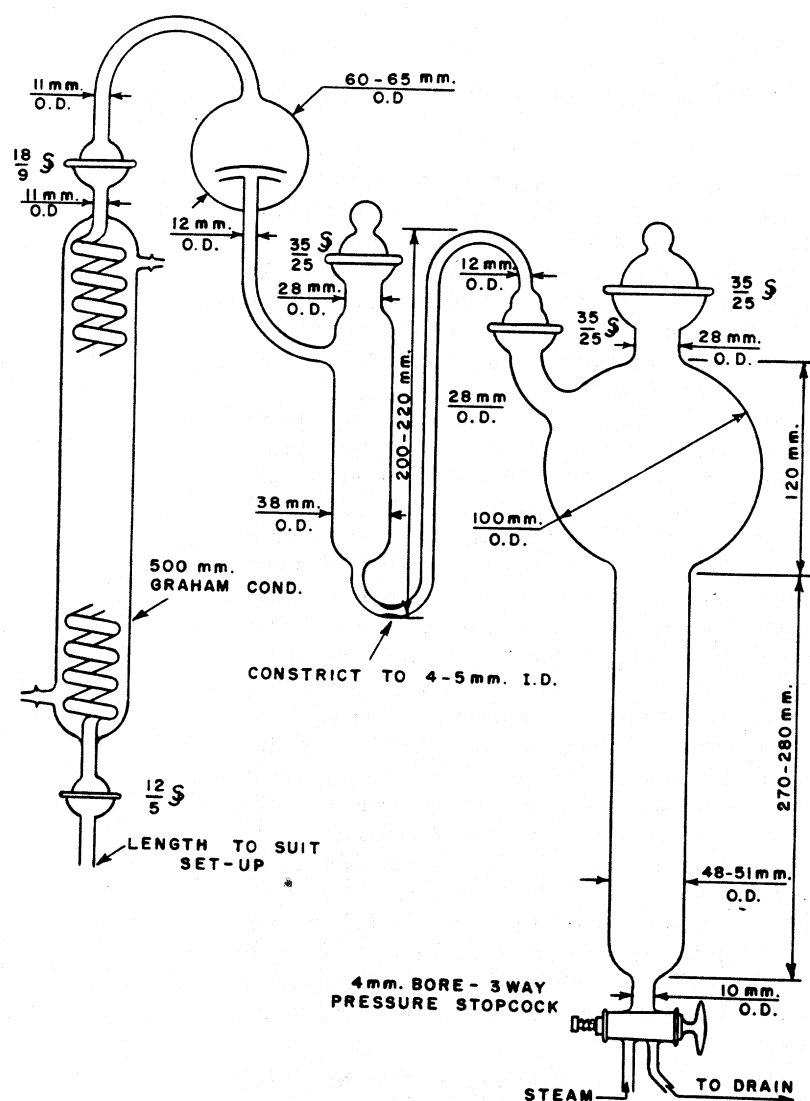


Fig. 1—Steam distillation apparatus.

in solutions containing both components, the absorbance due to BHT is calculated as the difference between the absorbance of the solution and that due to the BHA contained in the solution, Beer's law is then obeyed for BHT (last column, Table 1). The absorbance for BHT in the presence of BHA is almost twice that for BHT alone.

In the determination of BHA and BHT

in edible fats (1), *n*-butyl alcohol is added to the reaction mixtures just prior to the measurement of absorbance to eliminate turbidity due to steam-distilled fat. A secondary effect is to inhibit strongly further color development, thus permitting more accurate absorbance readings. It is for this latter effect that butanol is employed in the procedure described below.

METHOD

Reagents

(a) *Ethyl alcohol*.—95% (or 2 to 1 methanol-isopropanol solution).

(b) *Magnesium oxide suspension*.—Place about 2 g MgO (reagent grade) in a 500 ml narrow-mouth Erlenmeyer flask. Add 200 ml H₂O and shake to create milky suspension. Shake again immediately before adding to scrubber.

(c) *Borax buffer*.—Dissolve 2 g Na₂B₄O₇·10 H₂O in 100 ml water.

(d) *2,6-D reagent*.—Dissolve 0.010 g 2,6-dichloroquinonechloroimide in 100 ml 95% ethanol. Prepare fresh every other day and store in amber bottle.

(e) *2,2'-Bipyridine*.—Dissolve 0.100 g 2,2'-bipyridine in 1 ml 95% ethanol and dilute to 50 ml with water. Prepare fresh daily. (If kept in amber bottle and stoppered when not in use, it can be used for 2-3 days.)

(f) *Ferric chloride*.—Dissolve 0.200 g in 100 ml water. Keep in amber bottle and prepare fresh daily.

(g) *n-Butanol solution*.—Mix 2 parts *n*-butanol with 1 part 95% alcohol (v/v).

Apparatus

(a) *Spectrophotometer*.—Instrument capable of accurately measuring absorbance at 515 and 610 mμ.

(b) *Still*.—Fig. 1; 35/25 stoppers are Ace Glass Co. No. 825.¹

(c) *Steam generator*.—Electric pressure cooker with automatic control at 5 pounds steam pressure. (A Mirromatic¹ electric pressure pan with lid drilled and tapped for steam delivery tube has been found to be satisfactory.)

Distillation

Place about 135 ml of water in the still and add about 25 ml MgO suspension to the scrubber. Place a 250 ml volumetric flask, with mark at the 200 ml level, under the condenser tip. Introduce 20.0 ± 0.1 g potatoes into the still, stopper, and start steam flow immediately. Adjust steam flow to give approximately 20 ml condensate per minute. Distill 200 ml, turn off steam, and rinse condenser with about 40 ml 95% ethanol. Adjust flask to mark with 95% ethanol. Adjust distillate to temperature

¹ Mention of a specific company or product does not constitute endorsement by the Department over other companies or products not mentioned.

of standard solutions and start analysis within one hour.

Preparation of BHA and BHT Standards

Prepare a stock solution by dissolving 0.1000 g BHA in 95% ethanol; dilute to mark in a 100 ml volumetric flask, and mix. Prepare an intermediate solution by diluting a 10 ml aliquot to 100 ml in a volumetric flask with water. Prepare a standard solution by placing a 10 ml aliquot of the intermediate solution in a 250 ml volumetric flask, adding 190 ml water and bringing to mark with 95% ethanol. Prepare a standard solution of BHT in similar manner except that in preparing the intermediate solution, 50 ml of 95% ethanol must be added before dilution with water to keep the BHT in solution.

The standard solutions are stable for two days and the stock solutions for one week if they are kept refrigerated when not in use.

Analysis for BHA by Gibbs Method

Pipet 10 ml of about 20% ethanol into a small glass-stoppered bottle of 20 to 30 ml capacity and pipet 10 ml of distillate into a second bottle. Pipet 5 ml of about 20% ethanol and 5 ml BHA standard solution into a third bottle. Add 2 ml borax buffer, then 2 ml of 2,6-D reagent to each bottle and mix well. Measure the absorbance at 610 mμ in 1 cm cells *vs.* the blank in not less than 5 or more than 10 minutes after mixing. Calculate BHA content as follows:

$\text{ppm BHA} = A/B \times 25$, where *A* = absorbance of sample and *B* = absorbance of standard.

Analysis for BHT by the Emmerie-Engel Method

(Color reaction is light sensitive and consequently bottles used should be wrapped with black friction tape.)

Pipet 10 ml of about 20% ethanol into a taped 20 to 30 ml glass-stoppered bottle and pipet 10 ml of distillate into a second bottle. Pipet 5 ml of about 20% ethanol and 5 ml of BHA standard solution into a third bottle. Pipet 5 ml of BHA standard solution and 5 ml of BHT standard solution into a fourth bottle. Add 2 ml of the 2,2'-bipyridine reagent, then 2 ml of the ferric chloride reagent to each bottle and shake well. Let stand *exactly* 30 minutes, then add 5 ml *n*-butanol solution and mix well. *Exactly* 35 minutes after adding the ferric chloride reagent, start transferring the solutions to 1 cm spectrophotometer cells.

Measure absorbance at 515 $m\mu$ of each solution *vs.* the blank at exactly 38 minutes after adding the ferric chloride reagent. Calculate BHT content as follows: $ppm \text{ BHT} = \frac{C - (A \times D)/B}{E - D} \times 25$, where *A* and *B* = absorb-

ances of sample and BHA standard, respectively, with Gibbs' reagent (above), *C* = absorbance of sample, *D* = absorbance of BHA standard, and *E* = absorbance of BHA-BHT standard.

The absorbance of the BHA standard by the Emmerie-Engel method need be determined only when a new reagent is prepared. The absorbance of the BHA standard by the Gibbs method and of the BHA plus BHT standards by the Emmerie-Engel method should be determined simultaneously with each pair of samples.

When first using the method, calibration curves may be prepared to make certain that standards, reagents, and apparatus are all functioning properly. A straight line plot should be obtained.

There is a tendency for the cells to adsorb color bodies from the solutions containing ferric chloride. For this reason, cell corrections should be checked frequently and the cells cleaned with dilute HCl periodically.

Results and Discussion

The reproducibility of analytical values was checked with two samples of commercial flakes. As presented in Table 2, the data show that BHA, BHT, and the total can be determined with a precision better than ± 1 ppm.

The efficiency of the steam distillation was also checked as follows: Twenty grams of potato flakes, which contained no anti-

oxidants, were placed in the still and 130 ml water was added. Five ml of BHA and BHT intermediate solutions (0.5 mg BHA and 0.5 mg BHT) were added and steam distilled. Analysis of the distillates from three runs gave the following recoveries of antioxidants:

% BHA	% BHT	% Total
98, 93, 99	96, 93, 91	97, 93, 95

With flakes that had become slightly rancid it was necessary to remove interferences by steam distilling for two or three minutes before adding the antioxidants. Experience has shown that recoveries are significantly lower when more than one mg of antioxidant is present in the sample. Also, measurable blank values are obtained with untreated potato flakes and appreciable errors may occur at low levels of antioxidant. Analysis of potato flakes known to be free of antioxidants gave the following data:

BHA (ppm)	BHT (ppm)	Total (ppm)
0.3	2.8	3.1 (stored flakes)
0.7	1.9	2.6 (stored flakes)
0.0	2.0	2.0 (fresh flakes)

The method does not include corrections for blanks primarily because omission of the blank value approximately compensates for the small amount of antioxidants not recovered in the distillation step.

In this laboratory the house steam can be used for BHA and BHT analyses because it is sufficiently free of interferences. However, in some laboratories the use of house

Table 2. Antioxidant content of potato flakes

SAMPLE A			SAMPLE B		
BHA (ppm)	BHT (ppm)	Total (ppm)	BHA (ppm)	BHT (ppm)	Total (ppm)
26.4	10.3	36.7	25.5	6.9	32.4
25.9	10.7	36.6	24.7	7.6	32.3
26.2	11.5	37.7	25.0	7.1	32.1
26.2	11.5	37.7	26.0	5.6	31.6
25.7	9.9	35.6	25.7	6.5	32.2
25.0	11.1	36.1	25.7	7.4	33.1
25.3	11.1	36.4	25.7	7.4	33.1
Av. 25.8	10.9	36.7	25.5	6.9	32.4
S.D. \pm 0.5	0.6	0.8	0.5	0.7	0.5
Coeff. of Var. (%) 1.9	5.5	2.2	2.0	10.1	1.5

steam gave high BHT analyses. For this reason, an electric pressure cooker was adapted for use as a steam generator. Certain precautions should be observed in the use of a pressure cooker; it should be thoroughly cleaned and the rubber gasket should be soaked in alcohol prior to initial use. If the steam contacts rubber tubing high BHT values are likely to result; therefore a metal to glass connection must be made between the steam generator and the still.

It has been the procedure in this laboratory to give the still a special cleaning after about 25 analyses. Even though the still is rinsed after each analysis a residue from the potato flake samples builds up in time and can lead to significant blank values. This residue can be removed by back washing the still with a warm alcoholic KOH solution, followed by a dilute aqueous HCl solution, and finally rinsing with distilled water. The back washing or rinsing is done by allowing the suction created when the steam in the still condenses to draw the water or wash solutions backward through the still.

The presence of BHA appears to markedly reduce the stability of BHT in dilute solution. Consequently, standard solutions containing both BHA and BHT are not recommended because they are not stable and will lead to erroneously high BHT values when freshly distilled samples are analyzed. This instability is also the reason for specifying that sample distillates be analyzed within an hour after distillation.

Summary

A simple method for the rapid determina-

tion of BHA and BHT in potato flakes was developed by modification of a method applied to edible fats. The modifications consist of:

- (1) The use of an alkaline scrubber to remove volatile acidic interferences during steam distillation of the sample.
- (2) The determination of the absorbance of BHT in the presence of BHA.
- (3) Use of a rapid steam distillation apparatus which eliminates the need for superheated steam or for applying heat to the sample container.
- (4) Elimination of the use of absolute ethanol as solvent.

Acknowledgment

Dr. Robert L. Ferm of the Pillsbury Company and Mr. James Hale of the Borden Company provided the samples and participated in the collaborative study of the method. They obtained a similar order of precision in the analysis of BHA and BHT in these two samples. We are indebted to them, and to Dr. R. G. Buttery of the Western Utilization Research and Development Division, for testing the method and for suggesting refinements, particularly those concerning the stability of reagents and standards.

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